The use of innovative gadolinium-based contrast agent for MR-diagnosis of cancer in the experiment

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Abstract. The present study of the functional suitability and specific activity of the contrast agent gadolinium-based for magnetic resonance imaging demonstrated that the investigated contrast agent intensively accumulates in organs and anatomical structures of the experimental animals. In the model of tumor lesions in animals, study have shown that investigational contrast agent accumulates in the tumor tissue and retained there in for a long enough time.

1. Introduction
Modern technology of magnetic resonance imaging (MRI) is closely associated with the use of magnetic resonance contrast agents (MRCA), which not only increase the sensitivity and specificity of the MRI diagnosis, but also are an integral part of the diagnostic procedure. In European countries, the MRCA are used in 70-80\% of cases, in Russia this indicator is 9\%, primarily due to economic reasons and the lack of domestic contrast agents. The study of tumors without MRCA has low diagnostic information, so according to the WHO, at the MRI without contrast misdiagnosis rate may reach 40\%.The most frequently used in the practice of MRI paramagnetic metal is gadolinium (Gd + 3), which has seven unpaired electrons [1-6].

Solving the problem of creating a domestic gadolinium-based agent for magnetic resonance diagnostics will provide national health care effective MRCA cheaper foreign contrasts.

Therefore, the aim of the study was to assess the possibility of experimental use of a domestic contrast agent on the basis of a gadolinium complex "Pentagascan" for magnetic resonance diagnosis of malignant tumors.

2. Materials and methods
The MRSA ("Pentagascan" LLC "MedContrastSyntes") was studied. Agent is disodium gadolinium diethylenetriamine-N, N', N'', N"", -pentaacetic acid as a solution for infusion (Table. 1).
Table 1. Information about the "Pentagascan".

| Indicator                              | Regulated values                      |
|----------------------------------------|---------------------------------------|
| Appearance                             | Clear yellowish liquid                |
| Authenticity                           | Matches                               |
| pH                                     | 6.8–7.4                               |
| The color of the solution              | Not more Y5                           |
| Mechanical impurities                  | Absence                               |
| The relative density                   | 1.120–1.240                           |
| The content of total gadolinium, mg/cm³ | 75–90                                 |
| The content of the disodium salt of the complex gadolinium DTPA, mg/cm³ | 282–338                               |
| Excess DTPA, mg / cm³, not more than   | 10                                    |
| Free Gd+ of the total, %, not more than| 0.3                                   |
| Heavy metals, %, not more than         | 0.001                                 |
| Bacterial endotoxins, not more than    | 26 EU/cm³                             |
| Sterility                              | Sterile                               |

The first phase of the study was a comparative assessment of the suitability of the "Pentagascan" as MR contrast agent in intact animals. Comparison drug was "Magnevist". For this purpose 60 intact male rats white line "Wistar" weighing 300-350 g were studied. First, MRI was performed in all animals in native mode, without the introduction of paramagnetic agents. Then the animals were divided into two groups depending on the contrast agent. The study was performed in intact rats at 5 and 15 min after administration of the contrast agent into the femoral vein in a dose of 0.2 ml.

The second phase of the study was to evaluate the efficacy of "Pentagascan" in animals with experimental tumors. As a biological tumor model used malignancies transplanted mice - Lewis lung carcinoma. A solid version of Lewis lung carcinoma was used (Bank of cell lines RCRC. NN Blokhin, Moscow), supported by in vivo in mice lines C57B1 / 6j by intramuscular transplantation. To obtain tumor cells, mice were sacrificed by cervical dislocation. Tumor tissues were removed using sterile instruments, homogenized and counted in a cell concentrations Goryaev chamber. Cells were transplanted intramuscularly to the back the right paw at a concentration of 1-3 million cells per mouse.

All invasive procedures with animals were carried out with the use of inhaled or medication anesthesia. During this phase of the study 60 mice of the C57B1/6j were included in the experiment, at the time of the survey the mass of animals was 30-35, the average volume of the tumor node, measured instrumentally, was 2.3 ± 0.7 cm³.

All the animals were kept on a standard diet of the vivarium with free access to water, in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes.
Studies of mice with transplanted tumor were performed before and 1, 5 and 9 min after injection of contrast "Pentagascan" in a volume of 0.1 ml in the infraorbital sinus. In order to ensure complete immobility throughout the study experimental animals were anesthetized with an intramuscular infusion of 5% solution of ketamine.

Animal disposal on a pallet in a natural position. Centre tray with the animal was placed in a tunnel of the magnet isocenter, it used a 16-channel head coil HIGH-Head Matrix. The images were obtained with the following parameters Scan: T1-SE sequence - TR = 550 ms, TE = 17 ms, FoV - 181 × 270 mm, matrix - 292 × 512, slice thickness - 5 mm step - 1.5 mm; sequence of T1-TSE fatsat - TR = 677 ms, TE = 11 ms, FOV - 135 × 270 mm, matrix - 512 × 192, slice thickness - 3 mm, pitch - 0.3 mm.

Contrasting effect of the agent assessed visually and semi-quantitatively.

Statistical processing of the obtained data was performed using the software package STATISTICA. To estimate the differences of quantitative traits between groups a nonparametric Mann-Whitney test was used. Statistical significance of the dynamics of change in quantitative indicators was estimated using paired Wilcoxon test. In all procedures of the statistical analysis the significance level p was taken to be 0.05. Results are presented as M ± σ, where M is the arithmetic mean, σ - standard deviation.

3. Results and discussion

By visual evaluation of intact animal images after the administration "Pentagascan" and the post-injection "Magnevist" enhancement MR signal on post contrast scans in T1 images was marked. Contrast agent accumulated in the kidney cortex and vessels. On the delayed scans visualized homogeneous accumulation of contrasts in the kidney, liver and spleen. Also accumulation of paramagnetic in the bladder was determined (Fig. 1, 2).

![Figure 1](image_url)

Figure 1. Distribution of study drug ("Pentagaskan") in the body intact animals. After contrast injection modes T1-SE (a) and T1-TSEfatsat (b), there is its accumulation in renal parenchyma, liver, bladder.
The intensity of MR signal was measured built-in software module, by allocating ROI (region of interest) in the same area as a circle covering the cortex and medulla of the kidney. Considering the thickness of the slice, a volume of tissue in the region of interest was of 0.60-0.61 cm².

As a reference area used muscles (hip area). The MR signal intensity in the respective portions of the body are shown in Table. 2.

| Table 2. The intensity of the MR signal. |
|----------------------------------------|
| The value of the measured intensity in the selected areas of interest |
| (value / standard deviation) |
| Prior to the introduction | After contrast administration | Contrasted urine in the bladder |
| kidney | muscle | kidney | muscle | kidney | muscle |
| "Pentagaskan" T1-SE | 825.7/14.8 | 530.3/5.1 | 1902.3/28.7 | 565.8/10.3 | 2192.9/9.5 |
| "Magnevist" T1-SE | 864.4/54 | 520.9/14 | 2018.0/204 | 388.0/15 | 2142.0/446 |
| "Pentagaskan" T1-TSE fatsat | 879.8/40.1 | 580.6/83.1 | 2111.1/181 | 665.5/54.3 | 2188.2/34.4 |
| "Magnevist" T1-TSE fatsat | 928.7/80 | 680.8/25 | 1904.2/143 | 609.5/28 | 2291.3/751 |

The specific activity research study of "Pentagaskan" in intact animals indicate that contrasting ability of this MRCA does not differ from “Magnevist” the results of visual and semiquantitative evaluation.

In order to determine the optimal time intervals for the tumor study after intravenous contrast enhancement using a “Pentagascan”, it was carried out a series of experiments. The intensity and character of accumulation of contrast agent in tumor tissue at various time intervals: at 1, 5 and 9 min after intravenous contrast injection was visually evaluated. Time intervals are selected based on the intensity of metabolic processes in experimental animals.
It was noted that in the early phase "Pentagascan" rapidly accumulate in the renal parenchyma. The tumor tissue uptake of the MRCA has been observed in the early phase of the scan and stored in it for a long time. Intensity of drug accumulation in the tumor remained at the same level in different phases of the study (Fig. 3).

For confirmation of contrast enhancement of "Pentagaskan" quantitative parameters of the MR signal in the native phase of the study and after the intravenous administration of MRCA were calculated. The level MR signal in the tumor and in the kidney before and after intravenous contrast was measured. Mean values of MR signal intensity in tumor node and in the projection of the kidney are presented in Table 3.

Table 3. MR signal intensity in experimental animal studies in native phase and after contrast enhancement.

|                | Native phase | Contrast enhancement | Native phase | Contrast enhancement |
|----------------|--------------|----------------------|--------------|----------------------|
| **Kidney**     | 931.0/23.2   | 1929.0/16.9          | 561.9/11.2   | 1039.7/8.9           |
| **Tumor**      | 4.            |                       | 4.           |                      |

4. Conclusion
"Pentagascan" intensively accumulates in organs depleting drug that reliably fixed with any standard T1-weighted scans;
"Pentagaskan" intensively uptakes and stores for a long time in the pathological tumor tissue, which allows to perform quality MR scans of tumors.

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