ONCOLOGY

Evaluation of Magnetic Resonance Imaging Characteristics of New Nitroxyl Radicals on the Model of RLS Lymphoma

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Toxic and magnetic resonance contrast characteristics of new nitroxyl radicals Fur-135 and Fur-176 were studied in experiments on mice. The test compounds exhibited low toxicity and allowed us to increase contrasting of transplanted RLS lymphoma. Fur-135 differs by the type of contrasting from Gd^{3+}-containing preparation omniscan and locates the tumor focus with high precision.

Key Words: *magnetic resonance tomography; contrast agents; nitroxyl radicals; imaging; RLS lymphoma*

Visualization of pathological foci on T1-weighted images (T1-WI) is a priority problem of magnetic resonance imaging (MRI) [8]. Contrast agents on the basis of complex metal salts (Gd^{3+}, Fe^{3+}, Mn^{2+}) are currently used in MRI diagnosis for increasing the contrast of pathological areas [5]. One of the flaws of these compounds is their rapid elimination from the body, which shortens the time for obtaining contrast images. These agents can cause anaphylactic reactions [9] and complications in patients with renal and hepatic insufficiency.

Here we studied new agents from the group of organic metal-free paramagnetic compounds: nitroxyl radicals of the 2-imidazoline series containing imidazole-4-yl substitutes in the lateral chain characterized by high solubility and kinetic stability in water solutions [6].

The aim of this study was to evaluate toxic and imaging characteristics of two compounds from this group: Fur-135 and Fur-176 radicals previously studied *in vitro* [4].

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MATERIALS AND METHODS

Fur-135 and Fur-176 were synthesized at Laboratory of Multi-Spin Coordination Compounds of International Tomographic Center. Acute toxicity of Fur-135 and Fur-176 was studied on outbred male albino mice receiving single intragastric (0.5-5.0 g/kg) or intravenous dose (0.5-2.5 g/kg) of the agents. The mean lethal dose (LD_{50}) was determined by Kerber method.

Contrast characteristics of Fur-135 and Fur-176 were previously evaluated on 30 normal outbred male mice (25-30 g). Imaging characteristics of Fur-135 were studied in 60 male CBA mice (30 g) with transplanted RLS lymphoma. RLS malignant lymphoma [1] is a cyclophosphamide-resistant strain of LS mouse lymphoma [2]. By morphological signs this tumor is large B-cell non-Hodgkin lymphoma [3]. The choice of the model is explained by the importance of developing MRI contrast agents with better relaxation characteristics for detection of tumors of the lymphatic system at early stages of

development and dissemination. The agents were injected intravenously, the doses were chosen with consideration for the maximum imaging effect of the agent determined in previous experiments.

The animals from Laboratory of Experimental Animal Breeding (Institute of Cytology and Genetics) were kept under natural day/night regimen on standard granulated fodder.

RLS lymphoma was transplanted into the hip muscles (2×10^5 cells in 0.1 ml saline). Nine days after tumor transplantation the animals were divided into 5 groups. Mice of three groups received single intravenous injection of Fur-135 in saline in doses of 0.16, 0.32, and 0.48 g/kg. Animals of one group were injected with a Gd^{3+}-containing reference agent omniscan (Nycomed; 0.5 mmol/ml solution) in a dose of 0.1 mmol/kg (57.4 mg/kg), recommended for clinical diagnosis. Controls were injected with saline. The animals were sacrificed by cervical dislocation (5 animals per time point) 15, 20, 45, 60, 90, and 120 min postinjection. The animals were then positioned in a quadrature coil in a plane. MRI was carried out in the gradient echo modes (GEFI, T1-WI), RARE with short repeat time (Turbo-RARE, T2-WI), all with 1 mm^3 voxel, and in the myelography/urography mode (MYUR). The study was carried out in a Tomikon S 50 system (Bruker) at 0.5 T magnetic field strength and 15 mT/m gradient. The data were analyzed using ParaVision 3.0 software (Bruker). The T1-WI signal intensity was evaluated by densitometry of images using Image-Pro Plus 4.1 software (Media Cybernetics, L.P.). MRI contrast ($K_{1/2}$) of the studied area with adjacent tissue was calculated by the formula:

$$K_{1/2}=(S_1-S_2)/(S_1+S_2),$$

where S_1 and S_2 are signal intensities from different areas according to densitometry.

The liver, kidneys, spleen, and brain of animals were collected for histological verification of the pathology seen on tomograms. The organs were fixed in 10% neutral formalin and processed routinely on a MICROM histological complex (Zeiss). Sections (4-5 μ) were stained with hematoxylin and eosin, Schiff periodic acid, hematoxylin, and Orange G.

RESULTS

LD_{50} for Fur-135 and Fur-176 administered *per os* was >5 g/kg, which classifies these agents as low toxic substances. Being injected intravenously, they produced no lethal effect in doses of up to 1.8 g/kg.

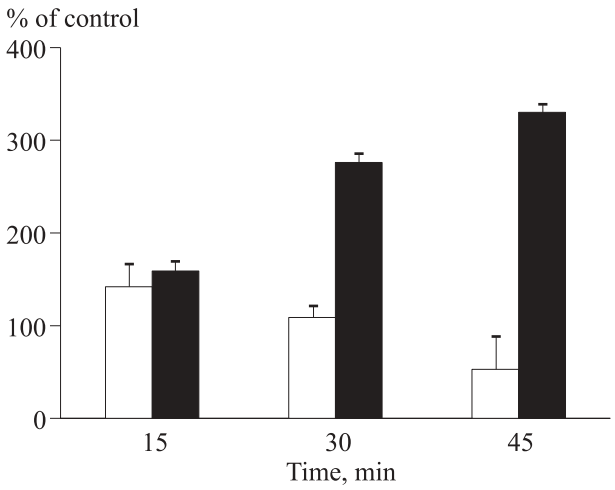

Fig. 1. Contrast between RLS tumor foci and adjacent muscle tissue after intravenous injection of Fur-135 in a dose of 0.45 g/kg (light bars) and omniscan (dark bars). Contrast in control animals is taken for 100%.

Imaging characteristics of Fur-135, Fur-176, and omniscan on T1-WI were first studied on intact animals. Fur-135 significantly amplified the signal from the urinary bladder without appreciably increasing the signal from other organs (Table 1). Fur-176 was effective for urinary bladder imaging and less so for hepatobiliary tissues. Omniscan injected intravenously amplified the signals from the heart, gallbladder, and urinary organs under the same conditions. Hence, nitroxyl radicals do not amplify the signals from normal tissues, in contrast to omniscan.

Visualization by contrast agents in animals with transplanted RLS lymphomas differed from normal. Intravenous injection of Fur-135 in a dose of 0.48 g/kg to mice with transplanted RLS lymphoma resulted in effective contrasting of the primary node within the first 15 min, which decreased by minute

TABLE 1. Visualization Characteristics of Fur-135, Fur-176, and Omniscan for Mouse Tissues after Intravenous Injection (%; *M*±*m*)

Organ	Maximum level of signal to T1-WI, %		
	Fur-135	Fur-176	omniscan
Heart	106±3	109±5	168±15
Liver	95±4	114±3	112±7
Gallbladder	116±12	128±6	178±8
Kidneys	101±4	99±3	152±16
Urinary bladder	326±130	186±25	276±35
Brain	104±4	102±3	98±8

Note. *$p<0.05$ compared to the control (100%).

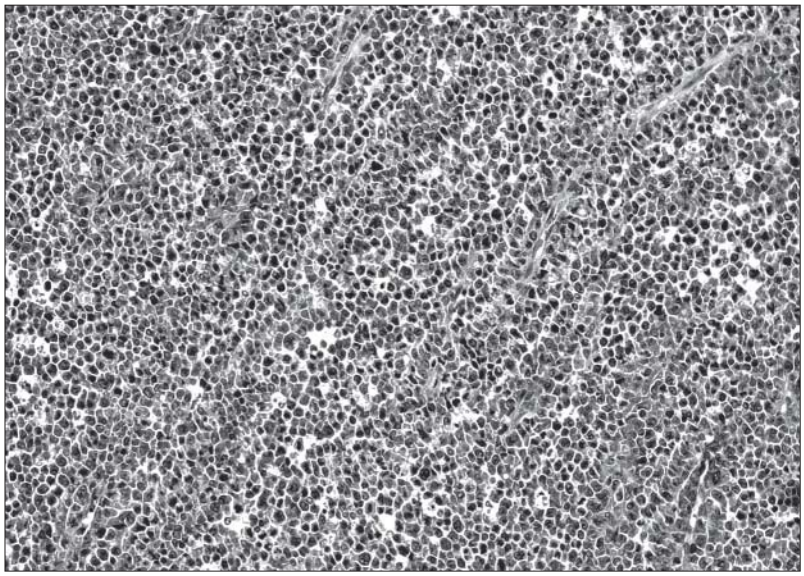

Fig. 2. RLS lymphoma primary node. Tumor emboli in capillary lumen. Hematoxylin and eosin staining, ×100.

45 (Fig. 1). Omniscan gradually increased the contrast over 45 min.

Fifteen minutes after injection the levels of contrast for Fur-135 and omniscan were similar, while later the dynamics of contrasting was opposite (Fig. 1). Longer contrasting of the tumor with omniscan was presumably due to more difficult penetration of the contrast agent into muscle tissue adjacent to the tumor. This could be caused by perifocal edema associated with high interstitial fluid pressure and impaired transport through the endothelial barrier [7]. By contrast, Fur-135 in tissues was reduced to diamagnetic hydroxylamine after 15 min, which led to a decrease of T1-WI signal.

Morphological study of the primary RLS lymphoma node showed the peripheral and central parts. Muscle tissue in the peripheral part of the tumor had typical structure with clearly discerned cross-striation. Vascular edema and plethora developed in the connective tissue. Tumor tissue grew into the muscle tissue as fine cords along the connective-tissue fibers. In the central part, the muscle tissue was completely replaced by the tumor tissue, which formed a node. Vast fibrinoid necroses of the tumor tissue developed in the center of the node. The tumor had no clearly discernible structure, but nodules of different shape and size formed round arterioles and venules. Tumor microemboli were detected in capillaries (Fig. 2). Qualitative evaluation showed that the location of foci detected by histological study correlated with the location of more intensive signal zones on MRI. The histological picture of the liver, kidneys, and spleen of animals showed metastatic involvement, presenting as infiltration paralleled by tissue edema and small focal necroses of individual cells. However, because of small size of the metastases they could not be evaluated by T1-WI densitometry, because the increase of the signal after injection of Fur-135 was negligible, while omniscan increased the signal intensity from the whole tumor (113-130% compared to the control).

Hence, intravenous injection of Fur-135 to mice increased MRI contrast of tumor foci without increasing contrasting of normal tissues. Omniscan increased contrasting of all tissues evenly as a result of agent diffusion from the vascular bed. Normally, nitroxyl radicals intensify contrasting of mouse tissues to a lesser degree than omniscan. Intravenous Fur-135 visualized RLS lymphoma, but was inferior to omniscan by the duration of contrasting.

The results indicate good prospects of searching for new MRI contrast compounds of other than metal nature among nitroxyl radicals of 2-imidasoline series and suggest a principal possibility for creation of an agent with the mechanism of action different from that of metal-containing agents.

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