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Study of Phthalocyanine Derivatives as Contrast Agents for Magnetic Resonance Imaging

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Abstract—The work is devoted to study of new prototype contrasting agents for magnetic resonance imaging on a base of manganese and gadolinium sulphophthalocyanines. Candidate complexes were shown to possess the R_1 molar relaxivity values comparable or exceeding ones for commercially available clinical contrast agents such as Magnevist. Intravenous administration of aqueous solution of studied complexes resulted in significant enhancement of contrast of C6 glioma imaging under T1-weighted protocol, allowing to consider them as perspective for further development. Near infrared photosensitizers based on nanoparticular forms of phthalocyanine derivatives.

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Conventional nuclear magnetic resonance imaging (MRI), within the scope of cancer diagnostics utilizes difference in the properties of tumor and nearby tissues, causing a change in the relaxation rates of water protons. Tumors of known localization on a background of nearby tissues, including adipose tissue, can be visualized by hardware and software methods that provide suitable contrasting for accurate diagnosis, however required manipulations are often time-consuming. At the same time, when tumor localization is not known or multifocal organ damage takes place, the whole-body magnetic resonance imaging must be performed. In this case, the hardware and software methods may not always provide a detailed examination of the patient within a time acceptable from the viewpoint of the diagnostic procedure.

One of efficient ways to resolve this problem of diagnostic MRI is based on use of contrast agents, substances able to accumulate in some tissue or organ structures, causing local change of relaxation rates and magnetic susceptibility. A tumor-tropic contrast agent is accumulated in tumor foci at larger extent than in adjacent normal (healthy) tissue, ensuring enhancement of water protons relaxation in these foci, resulting in a difference in signal and brightness of tumor image on the background of nearby tissue, i.e., providing a clearer visualization of the tumor.

Use of contrast agents can help not only to confirm the presence or absence of tumor and its location, but also to detect multiple metastases [1], which is not always possible using only the hardware and software methods.

Most first-generation contrast agents used in clinical oncodiagnostics are gadolinium chelate complexes. These substances commonly possess moderate tumor-tropic ability, resulting in need to use them at large diagnostic doses, on a background of general toxicity of all paramagnetic ions. Moreover, sufficient contrast enhancement with their use is observed only within the first 10–20 min post administration, not always allowing enough time for detailed examination.

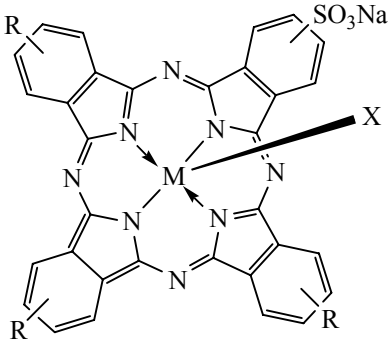
Recently, second-generation contrast agents have been developed based on Gd- and Mn-derivatives of tetrapyrroles, including bacteriopheophorbides [2] and phthalocyanines [3]. Other metal-complex derivatives of the same or similar structures were already shown to be successful as drugs for other modalities of tumor

diagnostics and therapy [2–5]. They can be selectively accumulated in tumors, which is presumably due to their affinity to low-density lipoproteins of blood plasma [4, 5].

In this work prototypes of domestic contrast agents based on derivatives of manganese and gadolinium sulphophthalocyanines were developed and studied for the magnetic resonance imaging of tumors.

Test Objects, Methods, and Equipment. Experimental Animals and Tumor Models

Authors have studied the sodium salts of manganese and gadolinium sulfophthalocyanines [$(SO_3Na)_{2,5}$PcMnX and $(SO_3Na)_{2,5}$PcGdX, respectively], prepared as stable mixtures of phthalocyanine complexes with a degree of sulfonation varied from 1 to 4 (the average degree of substitution ~2,5), and manganese tetrasulfophthalocyanine $(SO_3Na)_4$PcMnX.



R = H or SO_3Na, M = Mn or Gd, X = OAc or OH.

All test compounds, as water-soluble substances, were synthesized by metalation of corresponding sulfo-substituted metal-free phthalocyanines with metal acetates in a dimethylsulfoxide solution in the State Scientific Centre NIOPIK.

Sodium salts of composition $(SO_3Na)_{2,5}$PcMnX and $(SO_3Na)_{2,5}$PcGdX were synthesized from metal-free phthalocyanine sulfonic acid, obtained by sulfonation of unsubstituted metal-free phthalocyanine with chlorosulfonic acid in trichlorobenzene as a mixture of metal-free phthalocyanine sulfonic acids with varied degree of sulfonation.

For synthesis of tetrasulfo-substituted manganese phthalocyanine, $(SO_3Na)_4$PcMnX, we used, as starting compound, sodium salt of tetra-4(3)-sulfonic acid metal-free phthalocyanine obtained by demetallation of tetra-4(3)sulfonic acid zinc phthalocyanine, which in turn, was synthesized by fritting ammonium salt of 4-sulfophthalic acid with urea and zinc acetate [6]. Sulfophthalic acid was obtained by neutralization of 50% 4-sulfophthalic acid containing up to 25% of its 3-isomer with following evaporation.

The dynamics and selectivity of the accumulation of substances in tumor were determined on BDF_1 hybrid mice (female, 20–22 g body weight) bearing the P-388 limphocytic leukemia inoculated into right calf. By the beginning of the study, the tumor diameter reached about 1 cm. The accumulation of the contrast agent in the tumor and normal tissue was determined *in vivo* by diffuse reflectance spectroscopy [7, 8] on a LESA-01-BIOSPEC fiber optic spectrum analyzer (Russia).

The magnetic resonance images were obtained on Tomikon S50 and BioSpec 70/30 Bruker tomographs (Germany) at the magnetic field strength 0.5 and 7 T, respectively. A model tumor, glioma C6, was inoculated to 22 Wistar rats (male, body weight 220–250 g) intracranially (bypassing the gastrointestinal tract). The tomography was performed 14 days after inoculation. The substances studied were injected into the tail vein after dissolution in distilled water. The animals in the tomograph were immobilized using an isoflurane gas anesthesia mashine (UGO-Basile, Italy).

Relaxation Characteristics of Contrast Agents Based on $(SO_3Na)_n$PcMX

The molar capability R_1 of a contrast agent to enhance the relaxation rate was calculated by the least squares method as a proportionality coefficient between the change of the relaxation rate $1/T_1$ and concentration of the contrast agent:

$$R_1C = \frac{1}{T_1} - \frac{1}{{}^0T_1}, \quad (1)$$

where T_1 and ${}^0T_1$ are longitudinal relaxation times of water protons in the presence and in the absence of paramagnetic compound, respectively.

To determine the R_1 value, we prepared aqueous solutions of the test substances at concentrations ranging from 0.05 to 1.5 mmol/L. The solutions in special containers (1.5 mL) were put into the Tomikon S50 and Biospec 70/30 tomographs (with magnetic field of 0.5 and 7.0 T respectively). A sequence of electromagnetic 90° pulses was applied and the RARE VTR spin echo protocol with varied repetition times [9, 10] was used for recording signals.

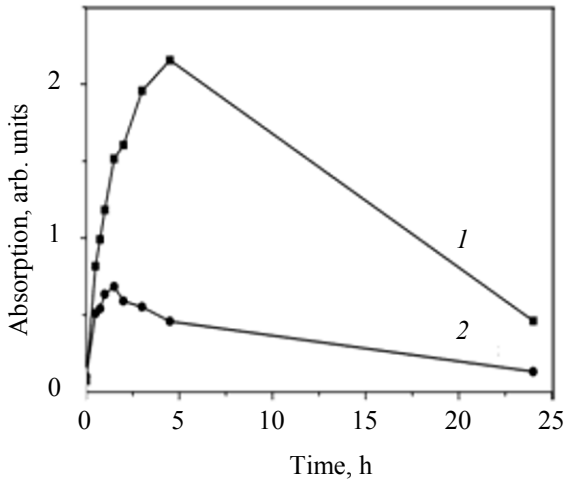


Fig. 1. Change in the $(SO_3Na)_{2,5}$PcMnX absorption with the time elapsed after the administration in (*1*) tumor and (*2*) normal tissue.

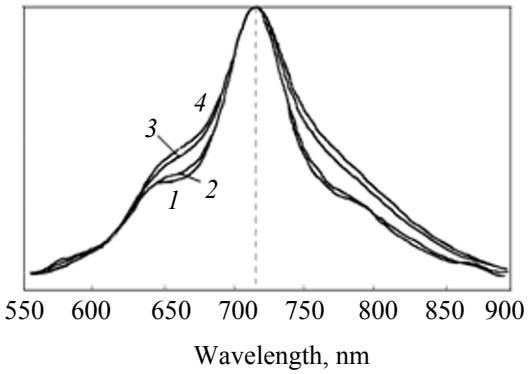


Fig. 2. The normalized absorption spectra of the $(SO_3Na)_{2,5}$PcMnX solutions of varied concentration. Concentration, mg/mL: (*1*) 0.005, (*2*) 0.05, (*3*) 0.01, and (*4*) 0.1.

In the equilibrium state of the spin system the net magnetization vector is parallel to the direction of constant magnetic field B_0 and is characterized by equilibrium magnetization $M_z(0)$ [1]. In this state, the value of the longitudinal magnetization M_z is equal to $M_z(0)$. If electromagnetic 90° pulses are applied after certain time intervals TR, then this magnetization M_z passes in the transverse XY plane and becomes zero. As a result of the relaxation process, the longitudinal magnetization M_z will return to its equilibrium value. In this case, the following relationship is satisfied [1]:

$$M_z(TR) = M_z(0)[1 - \exp(-TR/T_1)]. \quad (2)$$

In magnetic resonance imaging (MRI), the brightness of each point on the MR image is proportional to the intensity of MRI signal, which, in turn, is proportional to the magnitude of the longitudinal magnetization M_z. Therefore, in the presence of a certain concentration of the substance in the solution the experimentally obtained dependence of image brightness on TR was approximated by Eq. (2). From this dependence, we calculated the value of T_1 for a given concentration of the substance in a solution:

$$T_1 = -TR/\ln [1 - M_z(TR)/M_z(0)].$$

The R_1 values obtained at the magnetic field 0.5 and 7 T are collected in Table 1 for all substances tested. Data for the contrast agent Magnevist™ are given for comparison. The results obtained show that manganese and gadolinium sulfophthalocyanines are compable with Magnevist in relaxation rate enhancement and even surpass it, especially when measured in low-field tomographs. For low-field measurements, the R_1 increase is larger for manganese derivatives than for gadolinium complexes. This happens because the magnetic relaxation of manganese compounds largely depends on the scalar component, which is proportional to the magnetic field through the Larmor frequency [11]. Therefore, the R_1 factor for manganese compounds is strongly dependent on the magnetic field of the tomograph. Since Gd(III) ion forms ionic bonds, whereas protons are kept a large distance from the paramagnetic center via coordination bonds, the scalar spin pairing makes only negligible contribution to the total relaxation [9]. Therefore, for gadolinium compounds the contribution of scalar components can be disregarded.

Table 1. The relaxation enhancement R_1 (mol^{-1} s^{-1}) for paramagnetic substances of gadolinium and manganese sulfophthalocyanines

Magnetic field strength, T	$(SO_3Na)_{2,5}$PcGdX	$(SO_3Na)_4$PcMnX	$(SO_3Na)_{2,5}$PcMnX	Magnevist
0.5	8.14±0.12	7.8±0.2	7.3±0.2	5.6±0.2
7	7.5±0.2	5.12±0.18	4.0±0.3	4.8±0.2
R_1 (0.5 T)/R_1 (7 T)	1.09	1.52	1.83	1.17

Table 2. The signal ratio of the normal tissue to the tumor before and after administration of contrast agents based on gadolinium and manganese sulfophthalocyanines

Contrast agent	Signal		
	before administration	after administration	relative increase of signal
$(SO_3Na)_{2,5}PcGdX$	1.02	1.01	0.99
$(SO_3Na)_4PcMnX$	1.38	1.60	1.42
$(SO_3Na)_{2,5}PcMnX$	1.35	1.58	1.43

Choice of the Optimal Time of Magnetic Resonance Imaging

The optimal time of the magnetic resonance imaging can be determined by the dynamics of change of intensity of absorption of the contrast agent in the tumor and normal tissue, assuming that the tumor absorption has its maximum at the maximum concentration of the contrast agent. In turn, the dynamics of change of the optical absorption in tissues can be determined *in vivo* by diffuse reflectance spectroscopy (Fig. 1).

However, this method has its limitations. During first hours after the administration, the contrast agent resides mainly in the blood stream and forms aggregates owing to its high local concentration (about 0.15 mg/mL). In the presence of smaller number of non-aggregated molecules, the main absorption peak of the substance is widened and has reduced amplitude. As a result, in the initial stage after administration, the concentration of the contrast agent, as determined by the main absorption peak by the diffuse scattering method, may be underestimated. Therefore, the curve of absorption on the time elapsed after administration of the contrast agent passes a maximum by 2–4 h further than the true manganese concentration reaches the maximum value.

The assumption on the significant effect of aggregation on the absorption of manganese sulfophthalocyanines was tested. To do this, $(SO_3Na)_{2,5}PcMnX$ was dissolved in 1.6%-intralipid, an aqueous suspension simulating the scattering properties of the human skin. The $(SO_3Na)_{2,5}PcMnX$ concentration was varied from 0.005 to 0.1 mg/mL. As seen from Fig. 2, at the high concentrations of $(SO_3Na)_{2,5}PcMnX$ the absorption spectrum is diffuse and maxima of the aggregates are shifted relative to the main maximum.

In vivo measurement of the longitudinal relaxation time T_1 in tissues of experimental animals with tumors before administration of contrast agent and at different times after administration gives more reliable values to estimate the optimum time range for magnetic resonance imaging. Dynamics of changes in T_1 in C6 glioma and adjacent healthy brain tissue after administration of the studied compounds is shown in Fig. 3. As seen, T_1 in C6 glioma decreases by 35–40% after 20–50 min and then remains constant for more

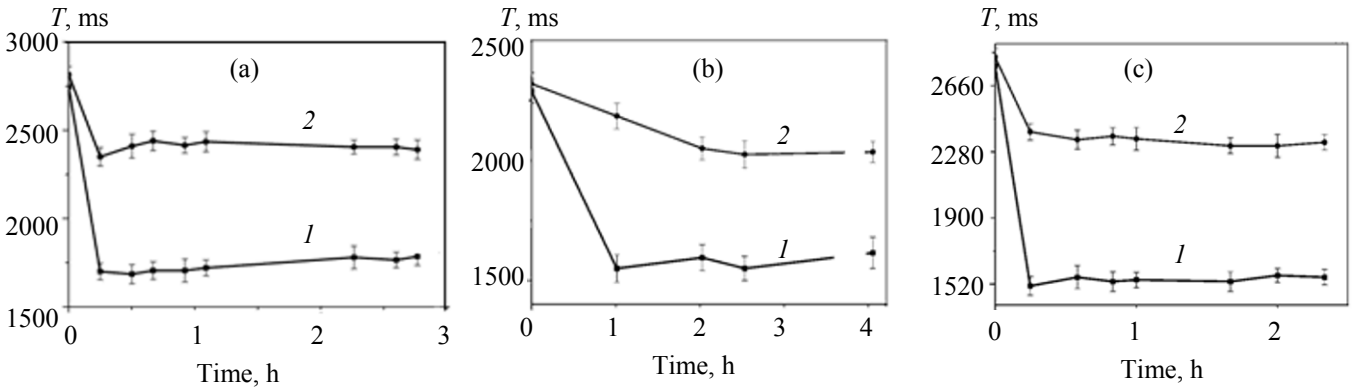


Fig. 3. Change in the longitudional relaxation time T_1 (ms) with time T (h) in (*1*) C6 glioma and (*2*) surrounding tissue after the administration of aqueous solutions of the contrast agents. Agent, dose (mg/ kg of body weight of rats): (a) $(SO_3Na)_{2,5}PcGdX$, 120; (b) $(SO_3Na)_{2,5}PcGdX$, 40; (c) $(SO_3Na)_4PcMnX$, 70.

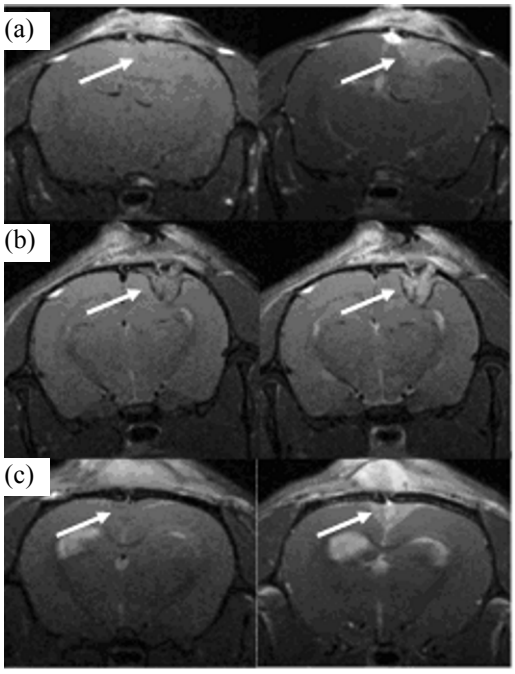


Fig. 4. Rat brain with C6 glioma (indicated by the arrow) in T_1–WI in the absence (left images) and in the presence (right images) of (a) $(SO_3Na)_{2,5}$PcGdX, (b) $(SO_3Na)_{2,5}$PcMnX, and (c) $(SO_3Na)_4$PcMnX.

than 2–3 h, whereas in healthy tissue the decrease is only 8–12%. This significant difference between the T_1 values in the tumor and normal tissues ensures bright signal of the tumor on T_1-weighted images, providing clear identification of its boundary and area (T_1–WI).

On MRIs obtained without a contrast agent the tumor signal was almost isointensive with adjacent tissue. However, after 120 mg $(SO_3Na)_{2,5}$PcMnX per 1 kg of body weight of the rat is administered, the hyperintense signal is displayed (Fig. 4a). This change of contrast is specific for the tumor, providing higher identification accuracy of its presence, location, size, and development, including invasion depth.

For the $(SO_3Na)_{2,5}$PcMnX и $(SO_3Na)_4$PcMnX concentrations 40 and 70 mg/kg of body weight of rats, respectively, the results were similar. On T_1–WI, C6 glioma is visualized as a bright area on the background of the surrounding benign brain tissue (Fig. 4).

Comparison of the magnetic resonance images obtained at different times after administration of Gd and Mn sulfophthalocyanines shows that the tumor contrast enhancement is provided for a sufficiently long time, at least 2 h (Table 2).

It should be noted also that the results on the contrast enhancement *in vivo* were obtained with the tomograph operating at the magnetic field 7 T. However, the effeciency of the contrast agent is dependent on the magnetic field. Consequently, the contrast of tumor image in a conventional clinical tomograph (magnetic fields mostly below 1.5 T) may be significantly larger. For example, in the presence of $(SO_3Na)_{2,5}$PcMnX, the signal ratio of the tumor to the normal tissue can be increased by 80%, which, in turn, will affect tumor contrast.

CONCLUSIONS

Gadolinium and manganese sulfophthalocyanines are comparable with commercial contrast agents based on Gd chelates (Magnevist, in particular) in relaxation rate enhancement and even surpass them under magnetic field of 0.5 T. Therefore, intravenous administration of aqueous solutions of Gd and Mn sulfophthalocyanines provides a significant contrast enhancement of C6 glioma on T_1-weighted images. Based on the data obtained, the above compounds can be classed as potential contrast agents promising for further research.

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