Doklady Chemistry, Vol. 404, Part 1, 2005, pp. 171–173. Translated from Doklady Akademii Nauk, Vol. 404, No. 2, 2005, pp. 198–200.

CHEMISTRY

Imidazol-4-yl 2-Imidazoline Nitroxide Radicals, a New Class of Promising Contrast Agents for Magnetic Resonance Imaging

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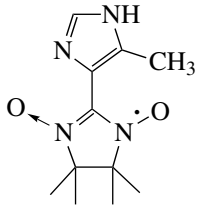
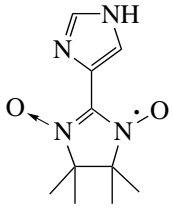
Received May 4, 2005

Recently, we developed methods of synthesis of an original group of 2-imidazoline nitroxides containing imidazol-4-yl substituents in the side chain (Fur-135 and Fur-176) and a series of their heterospin metal complexes [1, 2]. A specific feature inherent in Fur-135 and Fur-176 is their abnormally high solubility and kinetic stability in aqueous solutions. These facts prompted us to study the in-principle possibility of using these nitroxides as contrast agents for magnetic resonance imaging (MRI). Our study revealed the high efficiency of these compounds as contrast agents for generation of magnetic resonance (MR) images.



Fur-135



Fur-176

MRI visualization, which makes possible the rapid and noninvasive study of living tissues and the efficient visualization of pathological processes [3], is based on the difference in the spin–lattice ($T1$) and spin–spin ($T2$) relaxation rates of protons in biological tissues. Different pulse sequences used in MRI allow one to obtain images whose contrast is dominated by the $T1$ or $T2$ relaxation times [4]. At the same time, even the state-of-the-art technology does not always provide a sufficient level of visualization of the tissue to be studied. Special compounds are used for enhancing the signal from pathological foci. These compounds can be taken up into lesions and are able to enhance the contrast of $T1$- or $T2$-weighted images ($T1$-WIs and $T2$-WIs) [5, 6]. Basically, these are Gd^{3+} salts with polydentate ligands, such as deprotonated diethylenetriaminepentaacetic acid (the preparations Magnevist and Omniscan) [6]. Manganese and iron compounds (Teslascan, Abdoscan) are sometimes used. However, despite the high contrast of the images obtained with the use of these preparations, their application is associated with some risk. Cases of the development of anaphylactic reactions of the immune system and of kidney disorder have been documented [7]. For this reason, a paramagnetic metal ion that enhances the spin relaxation is, as a rule, introduced as a coordination compound with a high-denticity organic ligand. Such ligands should not only neutralize the charge on the metal ion but also firmly hold it in a complex since the human body has a very low tolerance for free transition metal ions [8].The thermodynamic stability of complexes can be enhanced only due to the chelate effect of polydentate ligands since, for rare-earth ions and high-spin Mn^{2+} and Fe^{3+} ions with the d^5 electronic configuration, the crystal field stabilization energy upon complex formation is zero [9]. However, the natural limitation on the number of coordination sites (no more than 6–9) puts a limit on the thermodynamic stability of these complexes. In addition, specially synthesized polydentate ligands, such as diethylenetriaminepentaacetic acid (H_5DTPA), are not metabolites and are foreign substances in living organisms.

An alternative approach to the solution of the problem of safe application of contrast agents can be the use of purely organic paramagnets based on nontoxic stable nitroxide radicals. An additional advantage of organic paramagnets is the possibility of modifying their structures with the objective of creating efficient MR contrast agents that can be selectively taken up into tissues [10]. In this context, since the advent of stable nitroxide radicals, numerous attempts have been made to use them as contrast agents. In in vivo studies, 1-pyrrolidinyloxyl [11, 12] and 1-piperidinyloxyl [13, 14] compounds have been used. However, it turned out that, first, the vast majority of available nitroxides were poorly soluble in water, which is an unfavorable factor for using a compound as a contrast agent. Second, those

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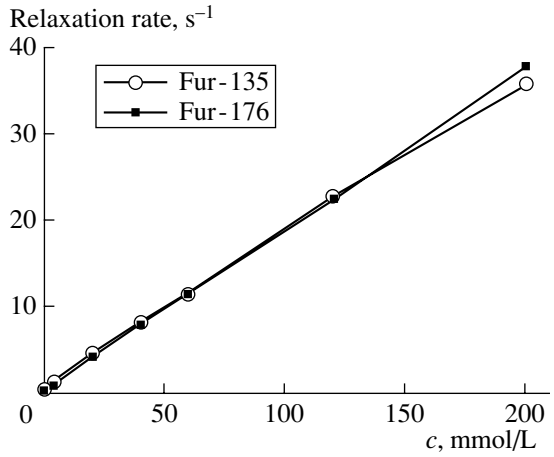


Fig. 1. *T*1 relaxation rate of aqueous solutions of Fur-135 and Fur-176.

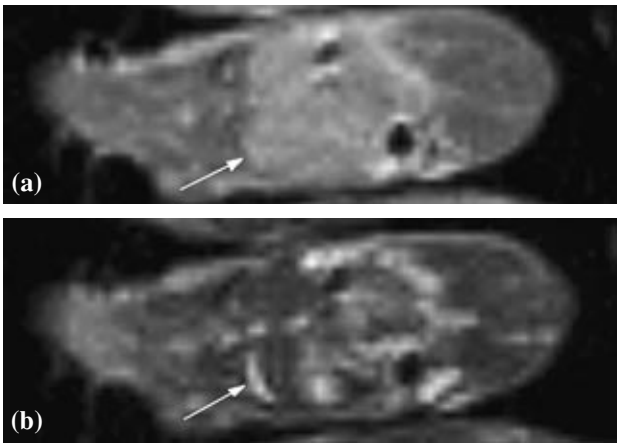
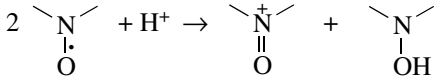


Fig. 2. Uptake of the Fur-135 nitroxide radical into a lesion focus (pleurisy, shown with an arrow): (a) *T*1-WI and (b) *T*2-WI.

nitroxides that were soluble to an extent either were unstable in aqueous solutions or were reduced in living tissues to the corresponding diamagnetic hydroxylamine derivatives or disproportionated in acid media [15] into an oxammonium salt and hydroxylamine, thereby losing their paramagnetism.



As is known, the pH in the human body varies from 1.4 in the stomach to 7.4 in the blood. Measurements of *T*2 relaxation times in aqueous solutions of Fur-135 and Fur-176 showed that these nitroxides are stable to both reduction and disproportionation in a wide range of pH. Variation in pH did not alter the relaxivity of these compounds both in freshly prepared solutions and after a lapse of 5–7 weeks. This is evidence of the kinetic stability of solutions, which makes these nitroxides suitable for both injection and enteral administration.

Experiments in outbred male mice showed that, for Fur-135 and Fur-176 administered enterally, the LD_{50} exceeds 5000 mg/kg, which corresponds to class IV of low-toxicity substances suitable for in vivo studies. The *T*1 and *T*2 relaxation times of aqueous nitroxide solutions were measured in the pharmacological concentration range on a Bruker Tomikon S50 instrument in a field of 0.5 T using the IR (TR = 5000 ms, IT = 10–600 ms) and MSME (TR = 5000 ms, TE = 16 × 15 ms) sequences. The in vivo signal intensity was quantified by densitometry on *T*1-WIs (GEFI-3D Steady State, TR = 100 ms, TE = 7 ms). Both low nitroxide concentrations and ones exceeding the maximum permissible dose (1/10 of LD_{50}, i.e., 500 mg/kg) were used. As can be seen in Fig. 1, the nitroxide radicals under consideration have good relaxation characteristics in the range of concentrations suitable for in vivo administration (based on a nitroxide concentration in the bloodstream of <60 mmol/L).

The Fur-135 and Fur-176 nitroxides retain their relaxation properties in the blood. When the nitroxide radicals were intravenously administered to outbred male mice (1.5 months old), the signal in the blood progressively increased over 30–45 min (the vascular phase). Then, the preparation was taken up into the normal tissues of the brain, lungs, heart, kidneys, and liver and into the gall bladder lumen by diffusion (the tissue phase of contrast enhancement, which was detected as a signal buildup on a *T*1-WI).

Of crucial importance is the fact that Fur-135 and Fur-176 can accumulate in pathological foci. This is exemplified by the MR image of a mouse thorax after a single intravenous administration of Fur-135 (Fig. 2). The *T*1-WI (Fig. 2a) shows that the administered Fur-135 enhances the signal in the edematous–exudative focus (in the absence of a contrast agent, this region shows a low signal intensity). The uptake of the preparation is fast enough (at the 10th to 15th min after intravenous administration) and continues, at least, up to 60–120 min. On the *T*2-WI (Fig. 2b), the accumulation of fluid in the pleural cavity (pleurisy) is clearly visualized. The Fur-176 nitroxide is also taken up into the inflammation focus but is visualized somewhat more weakly on the *T*1-WI. The nitroxides administered to mice were excreted from the body through the liver and kidneys (predominantly) within several hours.

Thus, we found that an intrinsic feature of imidazol-4-yl 2-imdazoline nitroxide radicals is their extraordinary kinetic stability both in aqueous solutions and when these solutions are administered to a living organism. These nitroxides have low toxicity and can accumulate in pathological foci. These compounds are the first examples of organic paramagnets that can serve as efficient contrast agents for magnetic resonance imaging of living organisms.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 05–03–32095) and the Siberian Division of the RAS (grant no. 146).

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